Evaluation of miR-135a/b expression in endometriosis lesions

RAFAELLA PETRACCO[1,2], ANA CRISTINA DE OLIVEIRA DIAS[3], HUGH S. TAYLOR[4], ÁLVARO PETRACCO[1,2], MARIÂNGELA BADALOTTI[1,2], JOÃO DA ROSA MICHELON[1,2], DANIEL RODRIGO MARINOWIC[1,2,5], MARTA HENTSCHKE[1,2], PAMELLA NUNES DE AZEVEDO[1,5], GABRIELE ZANIRATI[1,5] and DENISE CANTARELLI MACHADO[1,5]

[1]School of Medicine, Pontifical Catholic University of Rio Grande do Sul; [2]Fertilitat Center of Reproductive Medicine, Porto Alegre, Rio Grande do Sul 90610-000; [3]Quatro G P&D Ltda., Porto Alegre, Rio Grande do Sul 90619-900, Brazil; [4]Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University, New Haven, CT 06520-8063, USA; [5]Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul 90610-000, Brazil

Received December 21, 2018; Accepted July 10, 2019

DOI: 10.3892/br.2019.1237

Abstract. The pathogenesis of endometriosis is not clear; however, microRNAs (miRNAs/miRs) are involved in the pathogenesis. miRNAs are short noncoding RNAs involved in post-transcriptional regulation of gene expression by silencing the expression of target genes. The expression of *miR-135a/b* is associated with endometrial receptivity and implantation; the expression is also associated with the expression of certain genes, including homeobox protein Hox-A10 (*HOXA-10)*. The present study investigated the expression of *miR-135a/b* in eutopic and ectopic endometrium tissues throughout the different phases of the menstrual cycle. Samples of ectopic endometriosis lesions and eutopic endometrium tissue from 23 patients who underwent laparoscopic surgery were obtained and analyzed. miRNA was extracted and the expression levels of *miR-135a/b* were determined by reverse transcription quantitative polymerase chain reaction assays using U6 as a housekeeping control. The expression levels of *miR-135a* and *miR-135b* in endometriosis lesions were decreased compared with the levels in endometrium tissue. However, *miR-135a/b* expression levels were increased in the secretory phase compared with the proliferative phase in endometriosis lesions. The increased expression of *miR-135a/b* during the secretory phase compared with the proliferative phase suggested that these genes serve a determinant role in the homeostasis of reproductive tissue. Therefore, the expression of genes may affect endometrial functioning, impairing embryo implantation.

Correspondence to: Dr Daniel Rodrigo Marinowic, Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Building 63, 6690 Avenida Ipiranga, Porto Alegre, Rio Grande do Sul 90610-000, Brazil
E-mail: daniel.marinowic@pucrs.br

Abbreviations: miRNA, microRNA; HOXA10, homeobox protein Hox-A10; RT-qPCR, reverse transcription quantitative polymerase chain reaction; RNA, ribonucleic acid

Key words: endometrium, infertility, molecular biology, microRNA-135a/b

Introduction

Since 1860, when endometriosis was first described, its etiology and pathophysiology have not been fully understood (1). Endometriosis is a progressive estrogen-dependent disease characterized by the presence of endometrial cells (epithelial and stromal) outside the uterus, most frequently occurring in the pelvic organs and the peritoneum. Although its prevalence is not clear, it affects 5-10% of women of reproductive age and up to 50% of infertile women (2-4). Pelvic pain is the most common symptom, associated with dysmenorrhea, dyspareunia, or even chronic pelvic pain, which are frequently associated with infertility.

Delayed diagnosis complicates disease management. For earlier detection and a therapeutic follow-up, the identification of non-invasive biological markers with good specificity for this disease is a promising strategy. In cases of endometriosis, environmental, hormonal, immunological and genetic components are involved (5). In addition, epigenetic factors and adult stem cells also affect disease pathogenesis (6,7). Estrogen receptor gene polymorphisms and various exon-deleted progesterone receptor mRNAs are also involved in endometriosis (8). Previous studies have indicated the presence of aneuploidies in chromosomes 11, 16, and 17 and the loss of heterozygosity in chromosomes 9, 11, and 22 in the eutopic endometrium of patients with endometriosis, suggesting the possibility of a genetic component associated with disease development and progression (9-12). Previous breakthroughs in genetic mapping for endometriosis have been described and this technique is gaining importance due to the potential to show molecular aspects associated with diseases (12,13).

MicroRNAs (miRNAs) are small, single-strand noncoding RNA molecules containing ~22 nucleotides, which are

essential for gene regulation and are able to simultaneously control numerous genes (14). miRNAs are associated with different physiological processes, including apoptosis, differentiation and hematopoiesis. Following the identification of the role of miRNAs in cell function, miRNA expression has been associated with a number of diseases, in particular various types of cancer (15). Recent research has indicated that miRNAs and their target mRNAs are differentially expressed in endometriosis and other disorders of the female reproductive system.

Impaired endometrial receptivity, abnormal uterine bleeding, and endometriosis are some of the disorders that may be associated to the alteration of cellular and molecular homeostasis of the endometrium. Indeed, the expression of different miRNAs in the female genital tract, in normal and pathological tissues, suggests an association with the physiopathology of numerous diseases, including endometriosis (16,17).

The difference in miRNA expression between eutopic and ectopic endometrial tissues reflects the differential expression of genes involved in cell adhesion, extracellular matrix remodeling, migration, proliferation, immune system regulation and other events directly associated with the establishment of endometriosis implants (18,19).

miRNA135 (*miR-135*) has two subtypes: 135A and 135B. Previously, we demonstrated an inverse correlation between *miR-135* and homeobox protein Hox-A10 (*HOXA10)* in the mid-secretory endometrium during the implantation window in patients with endometriosis, which may explain implantation failure and the higher incidence of infertility in these patients. *HOXA10* was abnormally regulated in the endometrium of women with endometriosis by both *miR-135a* and *miR-135b*, suggesting a mechanism for the differential expression of *HOXA10* in disease pathogenesis (20). In addition, *miR-135* is upregulated in distinct phases of the menstrual cycles and has cell specificity; alterations observed in the early proliferative phase were demonstrated to be associated with a defective implantation window (20,21). *HOXA10* expression is downregulated in endometriosis lesions compared with in the eutopic endometrium. Besides, the association between *HOXA10* and *miR-135a* and *miR-135b* has been detected in the eutopic endometrium. However, the expression of this specific miRNA has never been evaluated in the ectopic endometrial tissue.

The present study aimed to assess *miR-135a* and *miR-135b* expression levels in the ectopic and eutopic endometrium and throughout the menstrual cycle. We hypothesized that *miR-135a* and *miR-135b* would be upregulated in the proliferative phase in ectopic and eutopic tissues.

Materials and methods

Patients and tissue collection. A total of 31 patients who underwent surgery for endometriosis diagnosis or treatment were recruited between March 2013 and May 2014. The study was approved by Pontifical Catholic University of Rio Grande do Sul Ethical Committees (approval no. 228.944; Porto Alegre, Brazil). Following collection of written informed consent, excised endometriosis lesions and adjacent endometrial biopsies were obtained from women with both surgical and histological diagnosis of endometriosis. All endometrial samples were obtained using a Pipelle catheter® (Laboratoire CCD), and endometriosis lesions were excised during laparoscopy. Endometrium and endometriosis biopsies were placed in a tube containing RNAlater® (Ambion; Thermo Fisher Scientific, Inc.) and stored at -80˚C until further processing.

The diagnosis of endometriosis was confirmed by histology. All 31 patients included in the study cohort were healthy and did not have any other medical issues, with the exception of endometriosis and/or sterility. The patients included had mild or moderate (stages II-III) endometriosis (revised American Society of Reproductive Medicine, classification system, 1997) (22).

Exclusion criteria included: The use of hormonal medications (or supplements) within three months prior to surgery; pregnancy; cancer; endometrial pathologies including polyps and submucosal/intramural fibroids; and patients who did not know their last menstrual period or refused to participate in the study. Out of the 31 participants, 8 were excluded due to insufficient mRNA levels. Following the first analysis, the samples were divided according to the menstrual cycle as follows: Proliferative, day 1-14 (n=11); and secretory, day 15-28 (n=12), according to Noyes criteria (23).

miRNA analysis using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the tissue fragment (~1 cm^3) using TRIzol reagent® (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Quantification and quality of RNA extracted were analyzed by a fluorometer platform Qubit® 2.0 (Thermo Fisher Scientific, Inc.) by performing serial dilutions according to the manufacturer's protocol. The total RNA was transcribed to obtain cDNA using a poly (A) RT-PCR method through the NCode miRNA first-strand cDNA synthesis MIRC-50 kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. For miRNA expression analysis, a RT-qPCR technique was performed using an Applied Biosystems® 7500 RT-PCR platform (Thermo Fisher Scientific, Inc.). Complementary primers to the sequences of *miR-135a* (forward, 5'-CCAGGC TTCCAGTACCATTAGG-3'; and reverse, 5'-GTTTCCGAG AGAGGCAGGTG-3') and *miR-135b* (forward, 5'-GCTTAT GGCTTTTCATTCCT-3'; and reverse, 5'-GTGCAGGGTCCG AGGT-3') were used. The experiments were performed on individual plaques for each miRNA using the U6 as an endogenous expression control. The primers used for the U6 small nuclear miRNA were (U6 forward, 5'-CTCGCTTCGGCAGCACA-3'; and reverse, 5'-AACGCTTCACGAATTTGCGT-3').

qPCR was performed using Supermix SYBR-Green and ROX (Quatro G P&D Ltda.). A total of 25 ng of cDNA were used for a 50-μl total reaction, according to the manufacturer's protocol. The thermocycling conditions were initiated by uracil-*N*-glycosylase activation at 50˚C for 2 min and initial denaturation at 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec, 57˚C for 20 sec and 70˚C for 30 sec; the final extension was at 72˚C for 5 min. The threshold cycle (Ct) and melting curves were acquired by using the quantitation and melting curve program of the AB 7500 platform. The miRNA level of each sample was normalized according to *U6* expression. Relative miRNA level was presented using the $2^{-\Delta\Delta Ct}$ formula (24).

Table I. Day of menstrual cycle, phase and endometriosis-associated symptoms in the patient cohort.

Patient no.	Day of menstrual cycle	Cycle phase	Symptoms
1	7	Proliferative	Pelvic Pain
2	21	Secretory	Pelvic Pain
3	15	Secretory	Pelvic Pain and Sterility
4	6	Proliferative	Pelvic Pain
5	19	Proliferative	Sterility
6	30	Secretory	Sterility
7	1	Proliferative	Pelvic Pain and Sterility
8	20	Secretory	Pelvic Pain
9	5	Proliferative	Sterility
10	27	Secretory	Sterility
11	22	Secretory	Sterility
12	28	Secretory	Sterility
13	19	Secretory	Pelvic Pain
14	15	Secretory	Sterility
15	11	Proliferative	Pelvic Pain
16	16	Secretory	Sterility
17	23	Secretory	Sterility
18	6	Proliferative	Pelvic Pain
19	11	Proliferative	Sterility
20	3	Proliferative	Sterility
21	13	Proliferative	Sterility
22	10	Proliferative	Pelvic Pain and Sterility
23	19	Secretory	Sterility

Statistical analysis. Data are expressed as the mean ± SD. Statistical analysis was performed using paired Wilcoxon signed-rank test to compare the ectopic and eutopic endometrium samples, and an unpaired Mann-Whitney test was used for the comparison between different menstrual cycle phases. All the analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Population and samples. The mean age of the 23 enrolled patients was 32 years (standard deviation ±2.93; range, 24-40 years). Of these patients, 16 were nulliparous and 7 reported at least one pregnancy. There were no significant differences between age and parity between the proliferative and secretory groups. A total of 1 patient had the clinical suspicion of endometriosis for 10 years, 2 for 3 years, 3 for 2 years, 4 for 1 year, 3 for 3-9 months, and in 10 patients, the diagnosis was made during surgery. The primary symptoms for surgery were pelvic pain (30.4%), sterility (56.5%) or both (13.0%). Table I summarizes the cycle phase characteristics and endometriosis-associated symptoms of all patients in the study cohort.

Expression of miR-135a and miR-135b in ectopic and eutopic endometrium lesions. The expression of *miR-135a* and *miR-135b* was analyzed in ectopic and eutopic endometrial tissues, in the proliferative and secretory phases, from women with endometriosis. The secretory phase was not classified into different stages. The results indicated slight decreases in *miR-135a* and *miR-135b* expression levels in the ectopic endometriosis endometrium compared with the eutopic endometrium tissue [3.54-fold decrease ($P=0.353$) and 4.06-fold decrease ($P=0.305$), respectively; Fig. 1]. When analyzed according to the phases, the expression levels of *miR-135a* and *miR-135b* were decreased in the endometriosis lesions compared with the eutopic endometrium tissue in the proliferative [n=11; 1.7-fold ($P=0.25$) and 0.25-fold decrease ($P=0.32$), respectively; Fig. 2A] and secretory phases [n=12; 5.31-fold ($P=0.266$) and 8.14-fold decrease ($P=0.129$), respectively; Fig. 2B]. All differences between these groups were statistically non-significant.

Expression of miR-135a and miR-135b in different menstrual cycle phases. The expression levels of *miR-135a* and *miR-135b* were increased in the secretory phase compared with the proliferative phase in the endometrium tissue [10.8-fold increased ($P=0.057$) and 7.6-fold increased ($P=0.11$; Fig. 3]. In the endometriosis tissue, the expression levels of both *miR-135a* and *miR-135b* were significantly increased in the secretory phase compared with the proliferative phase [7.9-fold increased ($P=0.036$) and 9.7-fold increased ($P=0.005$; Fig. 3].

Discussion

To obtain a favorable environment for the intrauterine embryo, adequate hormonal signaling must be performed, both with

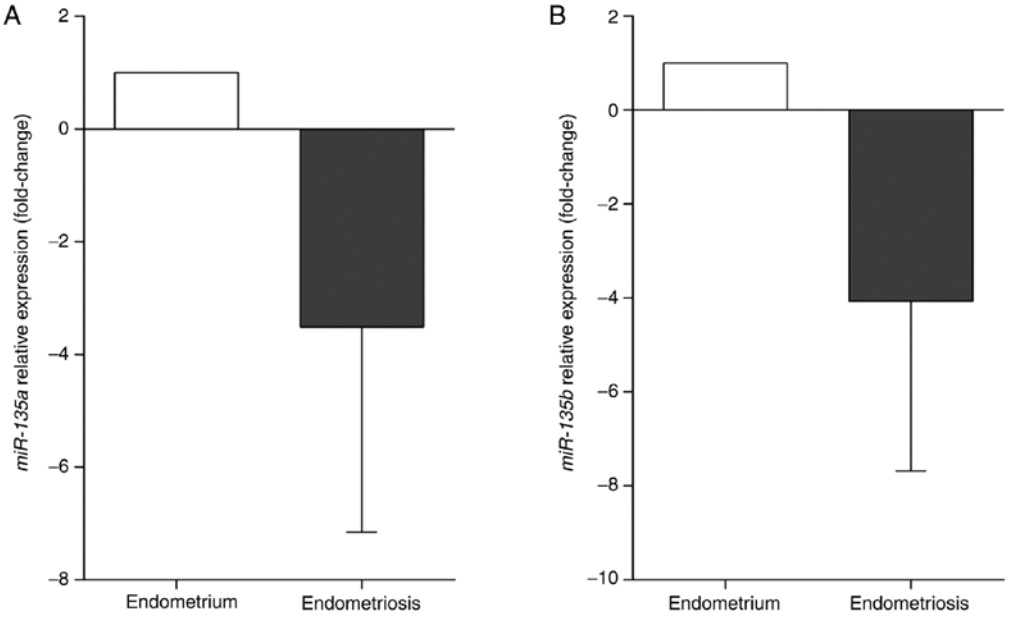

Figure 1. *miR-135a/b* expression in endometriosis. Expression of (A) *miR-135a* and (B) *miR-135b* in endometriosis and adjacent endometrium tissue samples. miR, microRNA.

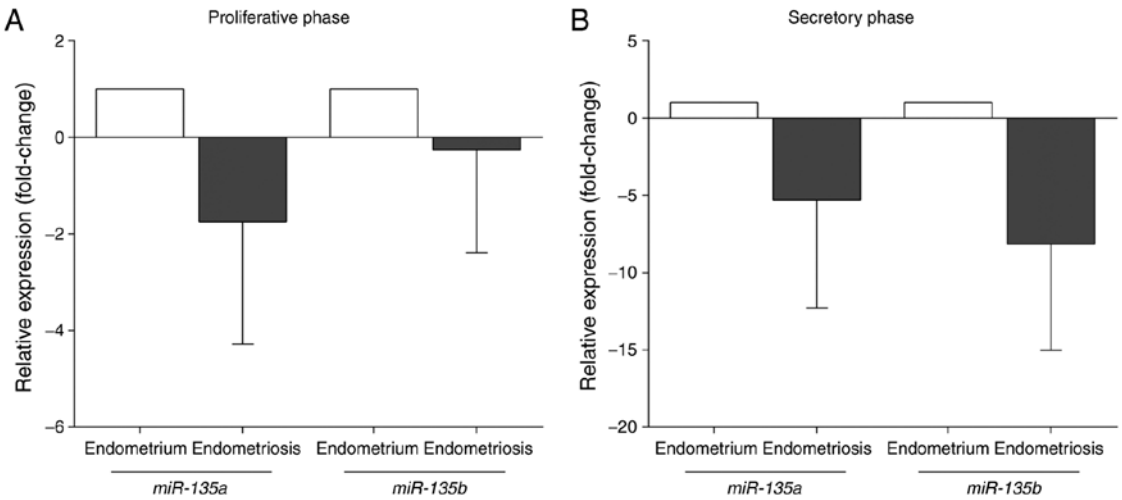

Figure 2. *miR-135a/b* expression in menstrual cycle phases. Expression of *miR-135a* and *miR-135b* in samples of endometriosis and adjacent endometrium tissue during the (A) proliferative and (B) secretory menstrual cycle phases. miR, microRNA.

endometrial and myometrial components. miRNAs are essential for normal uterine development and function.

To the best of our knowledge, the present study is the first to compare *miR-135* expression levels in the eutopic endometrium in patients with endometriosis in different menstrual cycle phases. We hypothesized that there would be an increase in miRNA expression in the proliferative phase in both types of analyzed tissues, but the increase was only significantly positive in endometriosis lesions.

Most studies have identified differential miRNA expression by comparing disease-free patients with those with endometriosis, and a number of studies have only analyzed the eutopic endometrium in a certain phase of the menstrual cycle (25-27). However, different endometriosis tissues, including those of endometrioma and peritoneal and ovarian endometriosis, express different profiles of miRNAs, and miRNA expression may or may not vary throughout the menstrual cycle phases (28). Ohlsson Teague *et al* (16) evaluated miRNA expression in different menstrual cycle phases and did not identify any difference compared with miRNA expression in the proliferative and secretory phases.

The difference between the studies may be explained by the fact that compared with the eutopic endometrium, the ectopic endometrium is probably susceptible to hormonal regulation; therefore, the lesions may suffer alteration regarding estradiol fluctuation. Estrogens and progestogens modulate chemotaxis and apoptosis in human endometrium and endometriosis tissues, and contribute to inflammatory responses, abnormal tissue remodeling, therapeutic refractoriness and disease persistence (29).

Progesterone has been suggested to control *HOXA10* expression, resulting in increased *HOXA10* levels in the

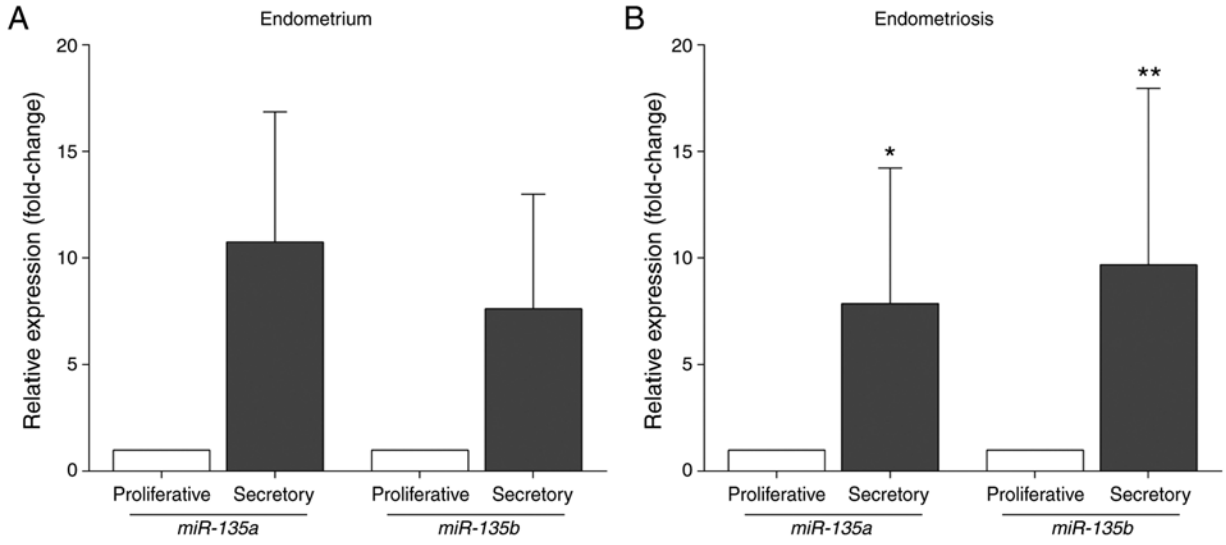

Figure 3. Cyclical *miR-135a/b* expression in healthy and endometriosis tissues. Expression of *miR-135a* and *miR-135b* during the different phases of the menstrual cycle in (A) healthy endometrial and (B) endometriosis tissues *P<0.05 and **P<0.001. miR, microRNA.

secretory phase of the menstrual cycle, also known as the window of implantation (30). However, Petracco *et al* (21) demonstrated that *HOXA10* expression was regulated by both *miR-135a* and *miR-135b* in the endometrium of females with endometriosis. Increased miRNA expression appeared to directly downregulate *HOXA10* expression, which is required for implantation in this group of patients.

In the present study, it was observed that, independent of the cycle phase, miRNA expression was always decreased in the ectopic tissue (endometriosis) compared with the endometrium tissue; however, this difference in expression was not statistically significant.

When *miR-135a* and *miR-135b* expression levels were compared separately in the eutopic endometrium and endometriosis lesions, between the secretory and proliferative phases, the expression levels were identified to be decreased in the proliferative phase. However, statistical significance was only observed in the analysis of endometriosis lesions. Therefore, the highest expression levels of *miR-135a* and *miR-135b* were identified in the eutopic endometrium tissue during the secretory phase. The addition of control samples from healthy individuals may improve the results of the present study, primarily to compare the relative expression levels of *miR-135a* and *miR-135b* in eutopic and ectopic tissues with basal levels in healthy tissues.

Naqvi *et al* (31) indicated that even distal endometrial lesions selectively and significantly altered the expression of genes, including *HOXA10* and progesterone receptors. The authors demonstrated that there is a uterine effect even when endometriosis is remote from the pelvis. Besides the decreased expression of *HOXA10,* the diminished progesterone receptor systemically induced by the remote disease may be associated with the increased levels of *miR-135a* observed in the secretory phase, when increased levels of progesterone are expected. The analysis of the association between miR-135 tissue levels and HOXA10 gene expression in eutopic and ectopic tissues may provide more conclusive results regarding the understating of endometriosis.

Santamaria *et al* (32) suggested that cells migrate from endometriosis lesions to the eutopic endometrium and disturb gene expression involved in embryo implantation and infertility. The ectopic cells migrate to the uterus and follow a different mechanism that alters uterine receptivity. The genes abnormally expressed in the endometrium of patients with endometriosis affect miRNAs and alter the effect of miR-135. In addition, Santamaria *et al* (33) in an extensive review regarding the roles of miRNAs in gynecological diseases revealed that 6 miRNAs have the potential to be used as new diagnostic tools. Certain miRNAs, if considered as a panel, exhibited a sensitivity of 96.6% and a specificity of 76.6% in blood endometriosis diagnostic tests (34). Therefore, these results additionally support the hypothesis that circulating miRNAs may be used as valuable non-invasive biomarkers for diagnosis and classifying different stages of endometriosis.

Cho *et al* (34) analyzed *miR-135* expression in the serum of patients with endometriosis and controls according to the menstrual cycle. They demonstrated that during the secretory phase, *miR-135a* expression was significantly decreased in patients with endometriosis compared with the controls (2.9-fold decrease; P=0.041). The discordance between serum and tissue levels of *miR-135a* expression may be due to the different expression of each miRNA and its tissue specificity, as miRNA expression patterns depend on the environment and specific tissue type. *miR-135* is expressed in the serum of patients with endometriosis and this is a promising result for the future of endometriosis research.

The present study demonstrated that *miR-135* is fully expressed in the ectopic and eutopic endometrium of patients with endometriosis. We hypothesize that the use of these data as diagnostic and therapeutic tools may lead to an improved understanding and management of endometriosis.

The roles of a number of miRNAs in the pathogenesis of endometriosis have been established. However, their exact contributions to the development and maintenance of endometriosis are not fully understood. Subsequent investigations are

mandatory for the development of diagnostic tools and therapeutic approaches that use miRNA manipulation technology to drive mRNA expression.

There are a number of pieces of evidence demonstrating that the physiopathology of endometriosis has a genetic component, and several studies have associated this disease with different miRNA levels and alterations in the endometriosis tissue (35,36). As the present study detected differences in the levels of *miR-135* expression during the menstrual cycle, it may be assumed that they serve a determinant role in the homeostasis of the reproductive tissue. Therefore, the profile of *miR-135* expression may affect endometrial functioning and impair embryo implantation.

Little is known about the regulation of miRNA expression, but it has been demonstrated that complex mechanisms are involved. miRNAs are expressed temporally depending on the stage of cellular development and at varied levels between different tissues (37). Therefore, miRNA expression is considered to be extremely specific and is highly regulated.

Although more studies using large sample sizes and healthy tissue as control groups are necessary to elucidate the whole function of *miR-135* in endometriosis, this miRNA is becoming an important target for the understanding of the pathogenesis of endometriosis and is a potential biomarker to guide diagnostic tools and therapeutic interventions. While other biomarkers for endometriosis are being studied, including matrix metalloproteinase (MMP)-2 and MMP-9, cancer antigen 125 remains the most recommended marker for suspicion of endometriosis and follow-up. The effects of miRNAs on the expression of genes involved in endometriosis pathophysiology in ectopic and eutopic tissues may be analyzed to elucidate the role of post-transcriptional factors in the genesis of this disease. There is an urgent requirement for a test based on non-invasive molecular biomarkers to identify the symptoms of endometriosis during the early stages of disease development.

Acknowledgements

No applicable.

Funding

The present study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior, Brazil (CAPES– Finance Code 001).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

RP, AP, MB, JdRM and MH were responsible for biopsy sample collection and clinical analyses. ACdOD, HST and DRM performed the molecular analyses, including miRNA extraction and RT-qPCR. PNdA and GZ performed the analysis and interpretation of data. DCM made substantial contributions to conception and design of the study, and the acquisition, analysis and interpretation of data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by Pontifical Catholic University of Rio Grande do Sul Ethical Committees (approval no. 228.944). Signed informed consents were obtained from the patients or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Benagiano G and Brosens I: History of adenomyosis. Best Pract Res Clin Obstet Gynaecol 20: 449-463, 2006.
2. Sensky TE and Liu DT: Endometriosis: Associations with menorrhagia, infertility and oral contraceptives. Int J Gynaecol Obstet 17: 573-576, 1980.
3. Houston DE: Evidence for the risk of pelvic endometriosis by age, race and socioeconomic status. Epidemiol Rev 6: 167-191, 1984.
4. Cramer DW and Missmer SA: The epidemiology of endometriosis. Ann N Y Acad Sci 955: 11-22; discussion 34-16, 396-406, 2002.
5. Giudice LC and Kao LC: Endometriosis. Lancet 364: 1789-1799, 2004.
6. Du H and Taylor HS: Contribution of bone marrow-derived stem cells to endometrium and endometriosis. Stem Cells 25: 2082-2086, 2007.
7. Lee B, Du H and Taylor HS: Experimental murine endometriosis induces DNA methylation and altered gene expression in eutopic endometrium. Biol Reprod 80: 79-85, 2009.
8. Kitawaki J, Kado N, Ishihara H, Koshiba H, Kitaoka Y and Honjo H: Endometriosis: The pathophysiology as an estrogen-dependent disease. J Steroid Biochem Mol Biol 83: 149-155, 2002.
9. Jiang X, Hitchcock A, Bryan EJ, Watson RH, Englefield P, Thomas EJ and Campbell IG: Microsatellite analysis of endometriosis reveals loss of heterozygosity at candidate ovarian tumor suppressor gene loci. Cancer Res 56: 3534-3539, 1996.
10. Gogusev J, Bouquet de Jolinière J, Telvi L, Doussau M, du Manoir S, Stojkoski A and Levardon M: Detection of DNA copy number changes in human endometriosis by comparative genomic hybridization. Hum Genet 105: 444-451, 1999.
11. Kosugi Y, Elias S, Malinak LR, Nagata J, Isaka K, Takayama M, Simpson JL and Bischoff FZ: Increased heterogeneity of chromosome 17 aneuploidy in endometriosis. Am J Obstet Gynecol 180: 792-797, 1999.
12. Albertsen HM, Chettier R, Farrington P and Ward K: Genome-wide association study link novel loci to endometriosis. PLoS One 8: e58257, 2013.
13. Fung JN and Montgomery GW: Genetics of endometriosis: State of the art on genetic risk factors for endometriosis. Best Pract Res Clin Obstet Gynaecol 50: 61-71, 2018.
14. Bartel DP: MicroRNAs: Target recognition and regulatory functions. Cell 136: 215-233, 2009.
15. Croce CM: Oncogenes and cancer. N Engl J Med 358: 502-511, 2008.
16. Ohlsson Teague EM, Van der Hoek KH, Van der Hoek MB, Perry N, Wagaarachchi P, Robertson SA, Print CG and Hull LM: MicroRNA-regulated pathways associated with endometriosis. Mol Endocrinol 23: 265-275, 2009.
17. Filigheddu N, Gregnanin I, Porporato PE, Surico D, Perego B, Galli L, Patrignani C, Graziani A and Surico N: Differential expression of microRNAs between eutopic and ectopic endometrium in ovarian endometriosis. J Biomed Biotechnol 2010: 369549, 2010.

18. Wu Y, Kajdacsy-Balla A, Strawn E, Basir Z, Halverson G, Jailwala P, Wang Y, Wang X, Ghosh S and Guo SW: Transcriptional characterizations of differences between eutopic and ectopic endometrium. Endocrinology 147: 232-246, 2006.
19. Eyster KM, Klinkova O, Kennedy V and Hansen KA: Whole genome deoxyribonucleic acid microarray analysis of gene expression in ectopic versus eutopic endometrium. Fertil Steril 88: 1505-1533, 2007.
20. Petracco RG, Kong A, Grechukhina O, Krikun G and Taylor HS: Global gene expression profiling of proliferative phase endometrium reveals distinct functional subdivisions. Reprod Sci 19: 1138-1145, 2012.
21. Petracco R, Grechukhina O, Popkhadze S, Massasa E, Zhou Y and Taylor HS: MicroRNA 135 regulates HOXA10 expression in endometriosis. J Clin Endocrinol Metab 96: E1925-E1933, 2011.
22. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 67: 817-821, 1997.
23. Noyes RW, Hertig AT and Rock J: Dating the endometrial biopsy. Am J Obstet Gynecol 122: 262-263, 1975.
24. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
25. Bjorkman S and Taylor HS: MicroRNAs in endometriosis: Biological function and emerging biomarker candidates†. Biol Reprod 100: 1135-1146, 2019.
26. Klemmt PAB and Starzinski-Powitz A: Molecular and cellular pathogenesis of endometriosis. Curr Womens Health Rev 14: 106-116, 2018.
27. Coutinho LM, Ferreira MC, Rocha ALL, Carneiro MM and Reis FM: New biomarkers in endometriosis. Adv Clin Chem 89: 59-77, 2019
28. Pan Q, Luo X, Toloubeydokhti T and Chegini N: The expression profile of micro-RNA in endometrium and endometriosis and the influence of ovarian steroids on their expression. Mol Hum Reprod 13: 797-806, 2007.
29. Reis FM, Petraglia F and Taylor RN: Endometriosis: Hormone regulation and clinical consequences of chemotaxis and apoptosis. Hum Reprod Update 19: 406-418, 2013.
30. Zanatta A, Rocha AM, Carvalho FM, Pereira RM, Taylor HS, Motta EL, Baracat EC and Serafini PC: The role of the Hoxa10/HOXA10 gene in the etiology of endometriosis and its related infertility: A review. J Assist Reprod Genet 27: 701-710, 2010.
31. Naqvi H, Mamillapalli R, Krikun G and Taylor HS: Endometriosis located proximal to or remote from the uterus differentially affects uterine gene expression. Reprod Sci 23: 186-191, 2016.
32. Santamaria X, Massasa EE and Taylor HS: Migration of cells from experimental endometriosis to the uterine endometrium. Endocrinology 153: 5566-5574, 2012.
33. Santamaria X and Taylor H: MicroRNA and gynecological reproductive diseases. Fertil Steril 101: 1545-1551, 2014.
34. Cho S, Mutlu L, Grechukhina O and Taylor HS: Circulating microRNAs as potential biomarkers for endometriosis. Fertil Steril 103: 1252-1260 e1251, 2015.
35. Borghese B, Zondervan KT, Abrao MS, Chapron C and Vaiman D: Recent insights on the genetics and epigenetics of endometriosis. Clin Genet 91: 254-264, 2017.
36. Krishnamoorthy K and Decherney AH: Genetics of endometriosis. Clin Obstet Gynecol 60: 531-538, 2017.
37. Neilson JR, Zheng GX, Burge CB and Sharp PA: Dynamic regulation of miRNA expression in ordered stages of cellular development. Genes Dev 21: 578-589, 2007.